# PRECESSION ENHANCED ELECTRON DIFFRACTION APPLICATIONS FOR TEM

Alan Robins
Director of Business Developement NanoMEGAS



Stavros Nicolopoulos
Thanos Galanis
Vrettos Stelliou
Spirros Pantaretos

# PRECESSION ENHANCED ELECTRON DIFFRACTION APPLICATIONS FOR TEM

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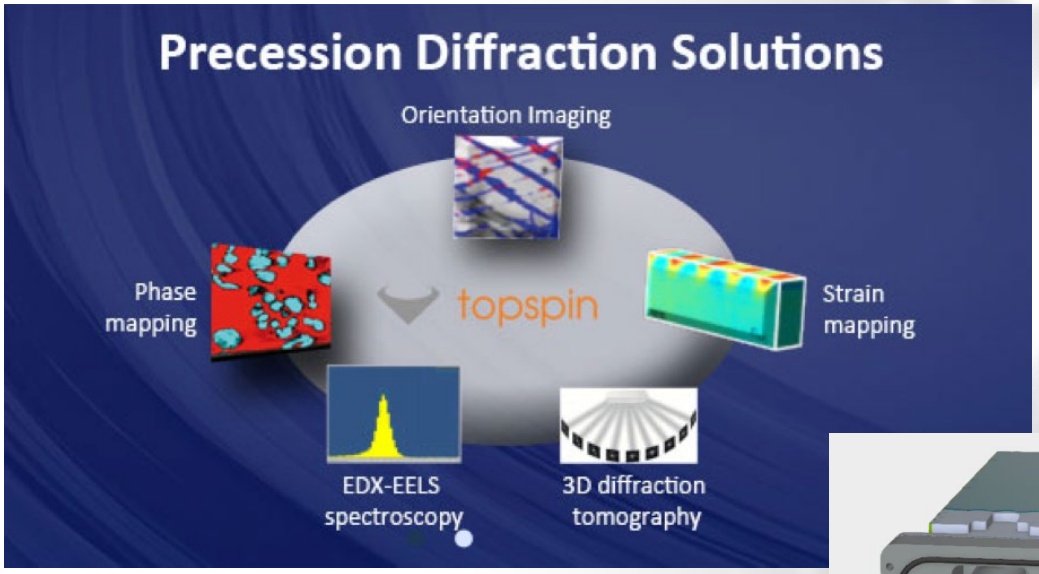
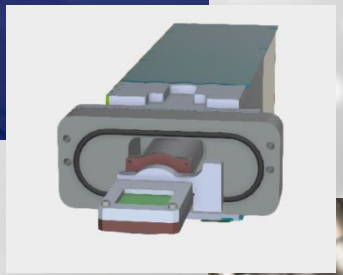
# ELECTRON DIFFRACTION AND PIXELATED DETECTORS

➢ 500 PED related publications



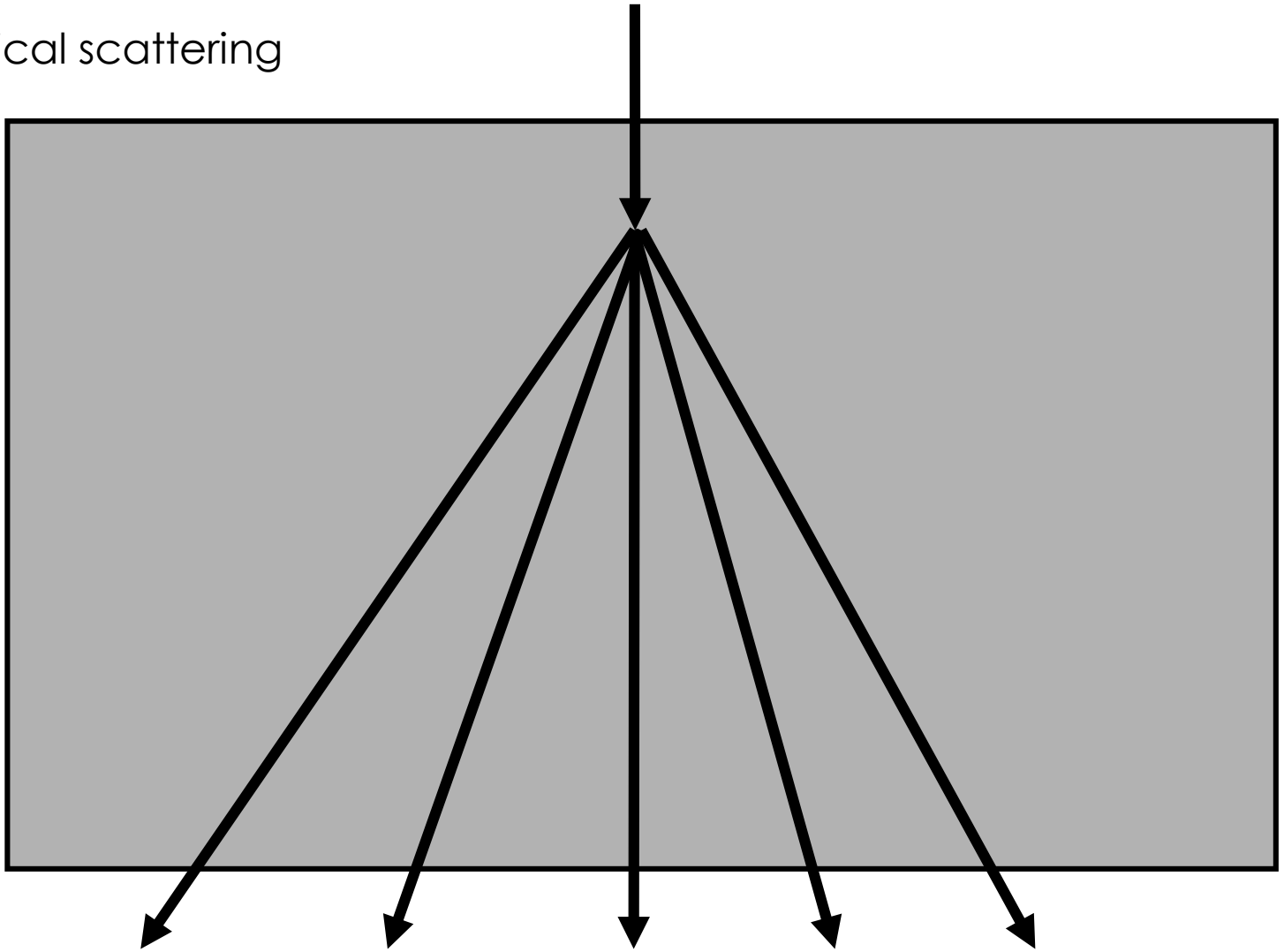



esteem3

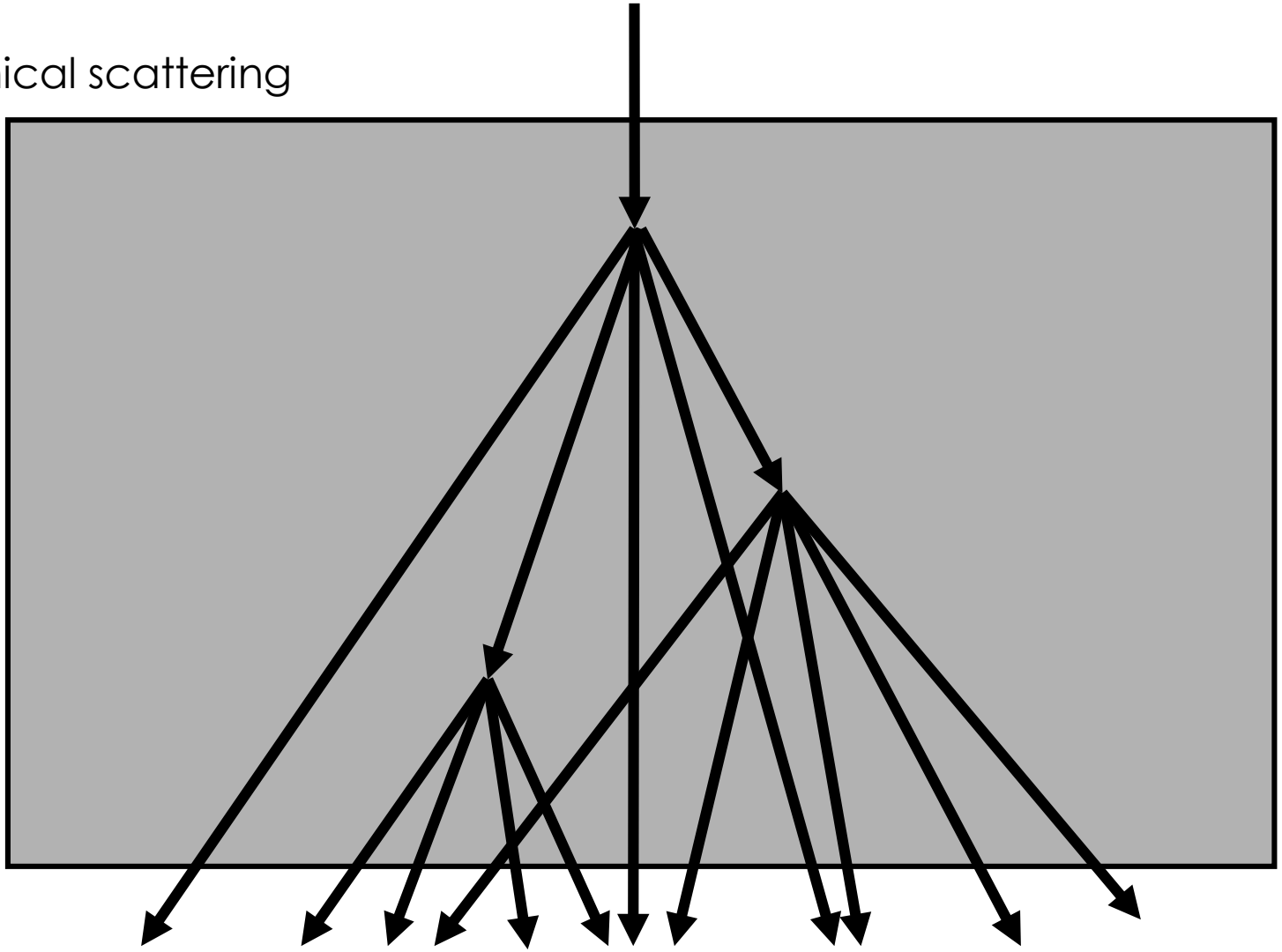
# Electron diffraction : Dynamical effects

Kinematical scattering



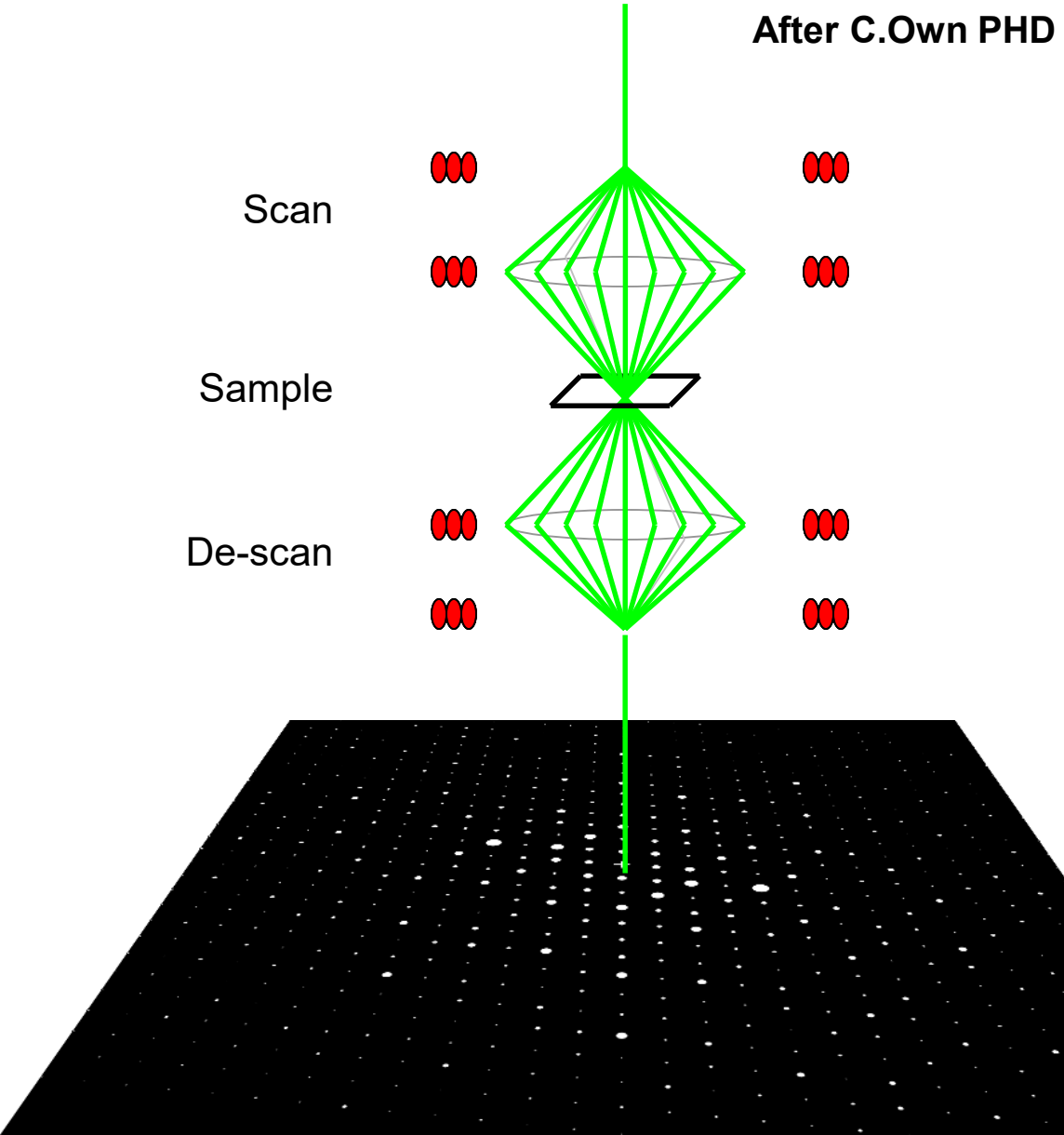
# Electron diffraction : Dynamical effects

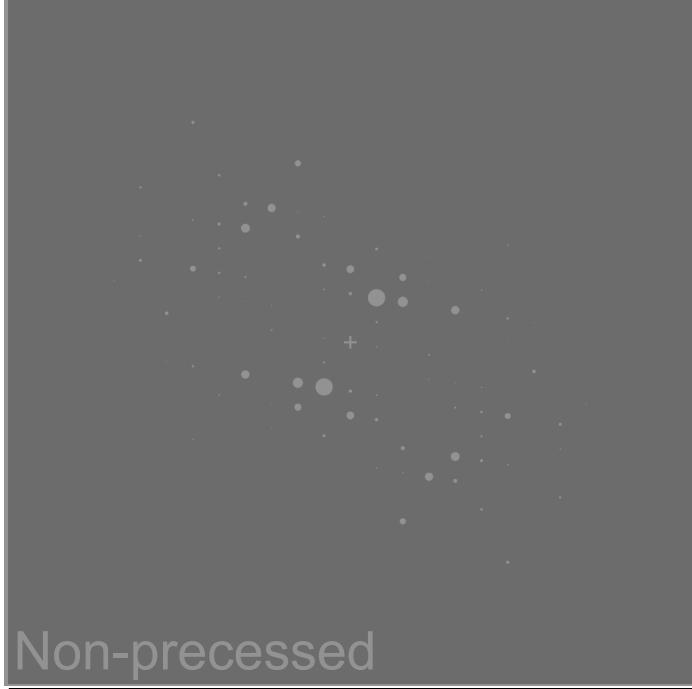
Dynamical scattering

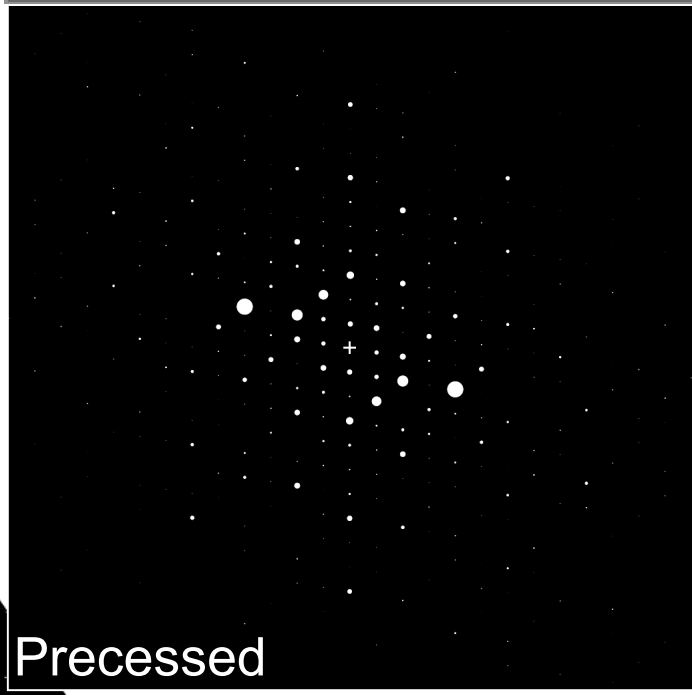


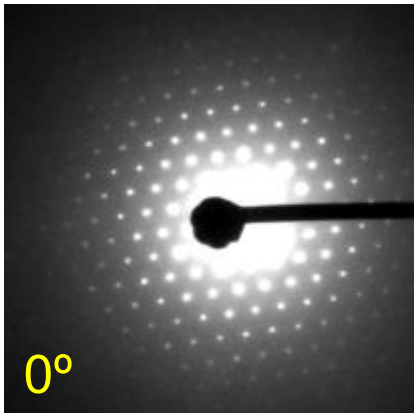
Courtesy Dr. E.Mugnaioli Univ of Sienna

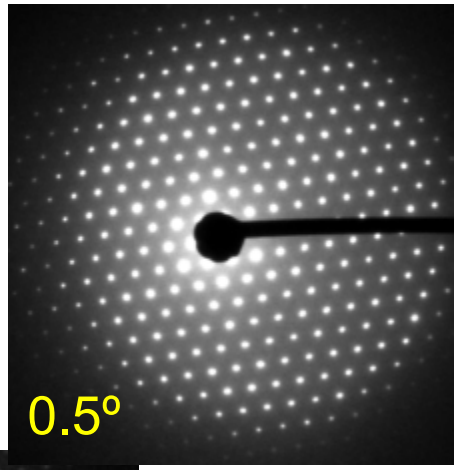
# Vincent-Midgley Precession Method

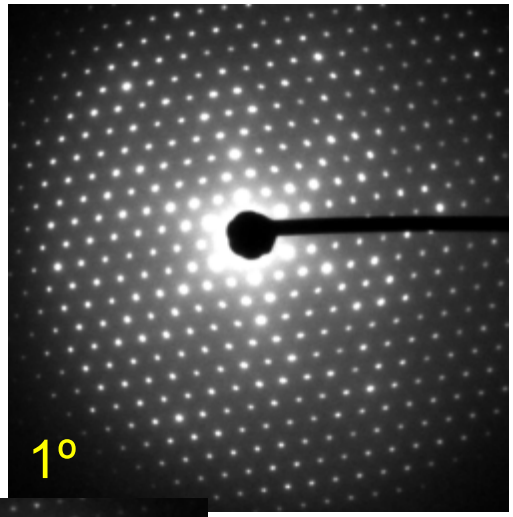


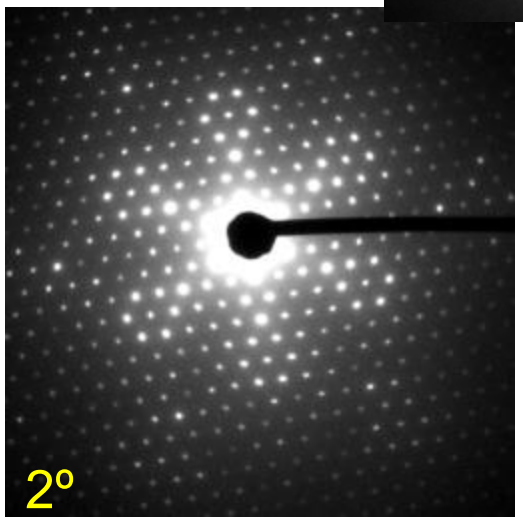



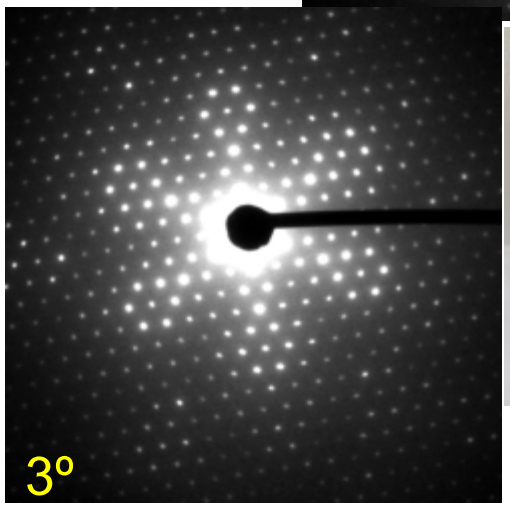





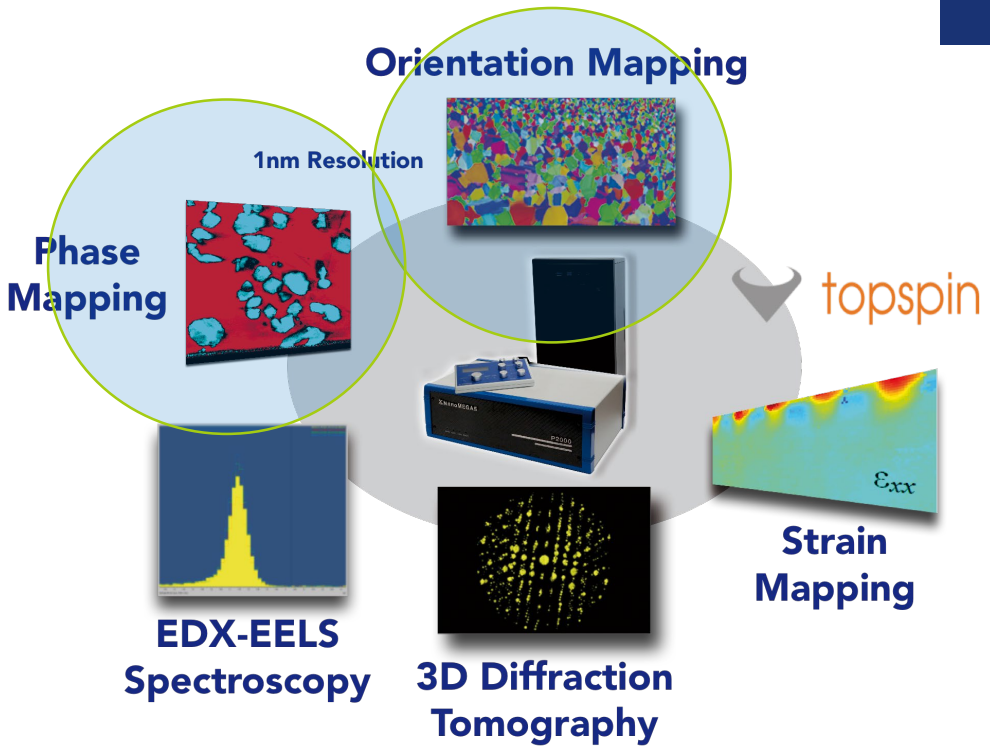




**Precession unit Digistar retrofitable in TEM**

# Orientation / phase mapping & Direct Detection Detectors

# ASTAR: TEM Orientation and Phase Mapping

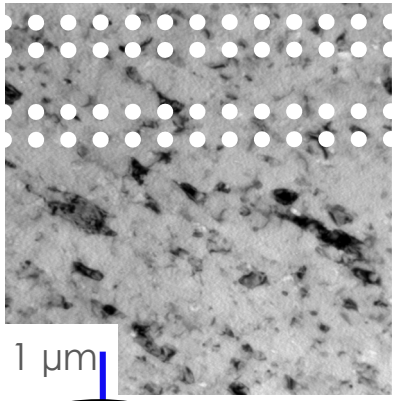
➢ 180 installations world-wide in TEM





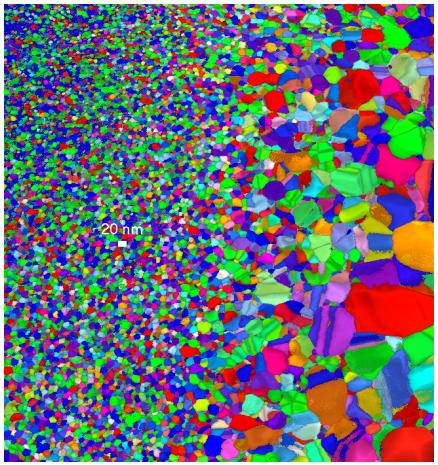
**ASTAR : EBSD type tool for TEM**

# ASTAR : Automated Crystal Orientation Mapping

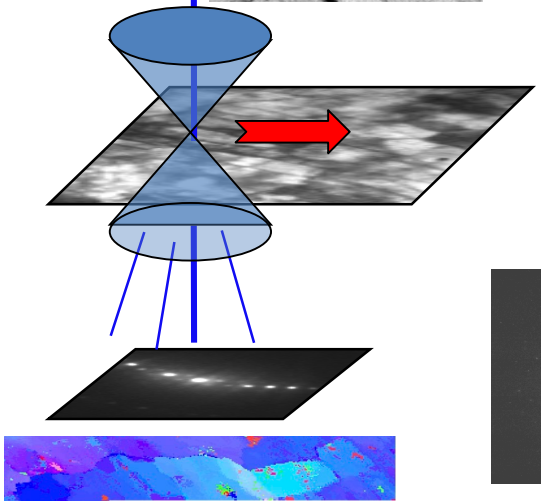


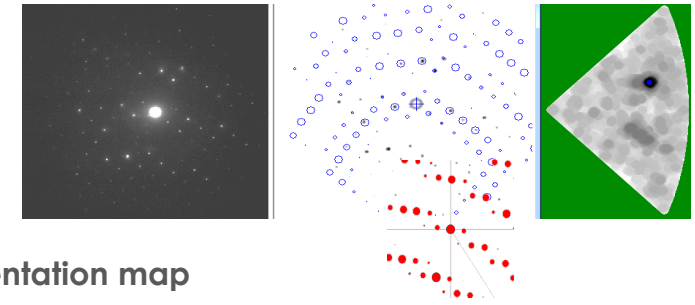
1 µm

Scan sample area




Orientation map

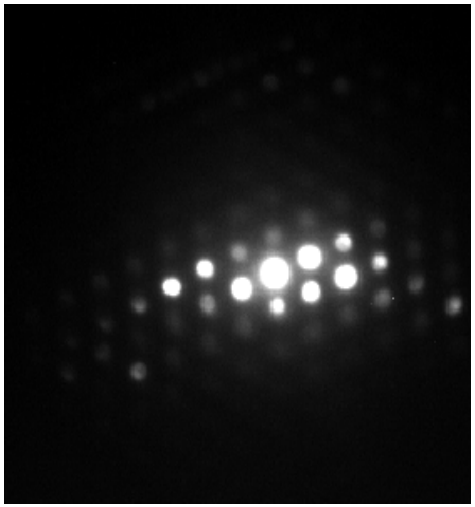




Orientation map

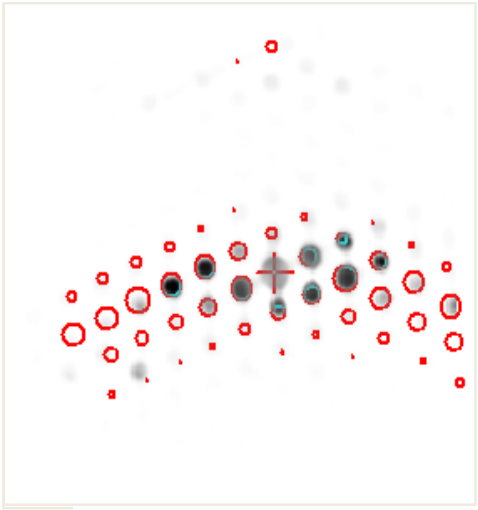
## ASTAR : EBSD type tool for TEM

# Identification example : nanocrystalline Cu

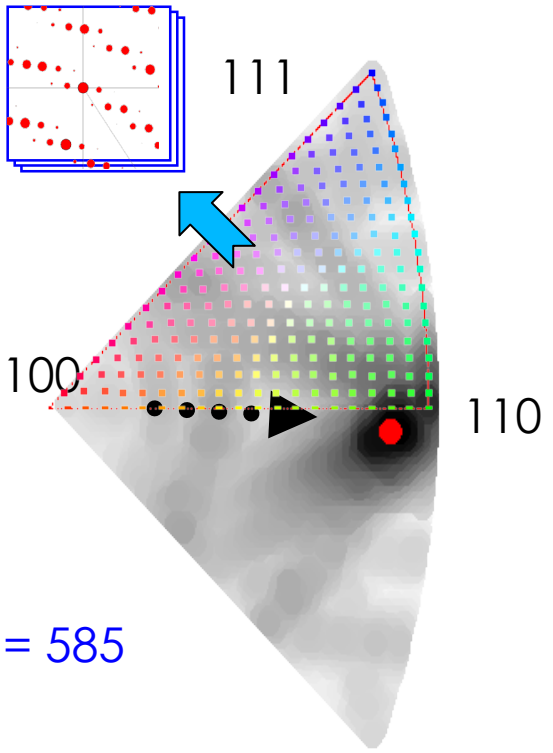


Diffraction pattern

**( nanocrystalline cubic copper)**



correlation index = 585

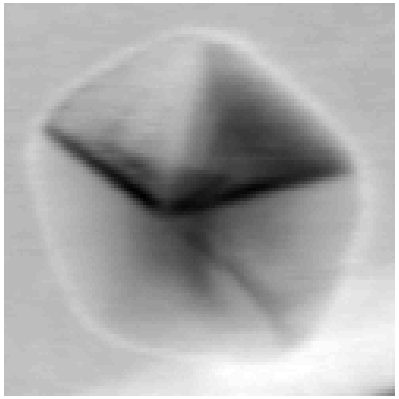



**Correlation index map**

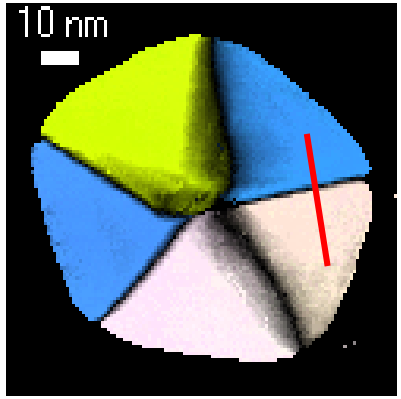
**For a given ED pattern, the correlation index map is calculated for all possible template orientations and plotted on a map that represents a portion of the stereographic projection (reduced to a double standard triangle). That resulting map reveals the most probable orientation for every experimental spot ED pattern ( in this case ED pattern is found to be close to 110 ZA orientation )**

# ASTAR : 1 nm spatial resolution orientation map

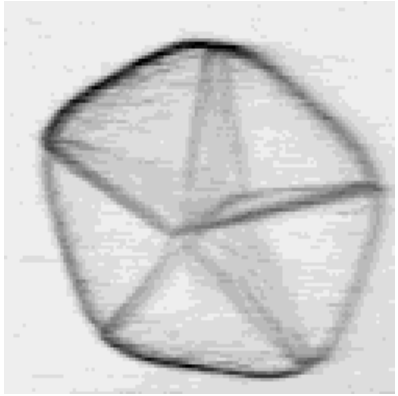
**Multi-twinned gold particles** (JEOL ARM 200)



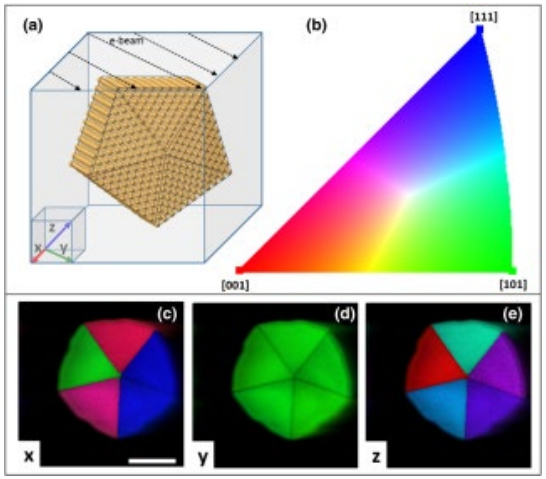
Virtual Bright-Field

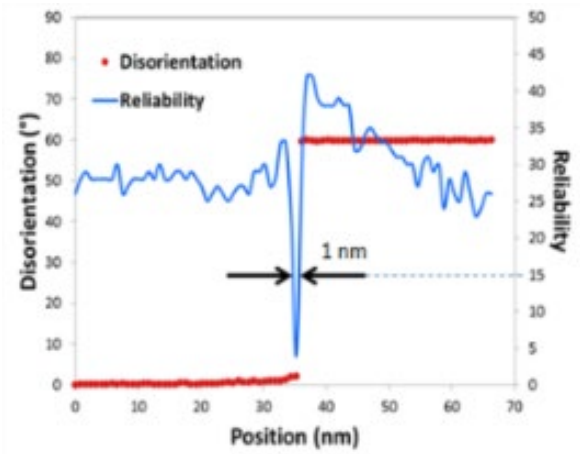



Orientation + Reliability



DP cross-correlation




**ASTAR : EBSD type tool for TEM**

NanoMEGAS
Advanced Tools for electron diffraction

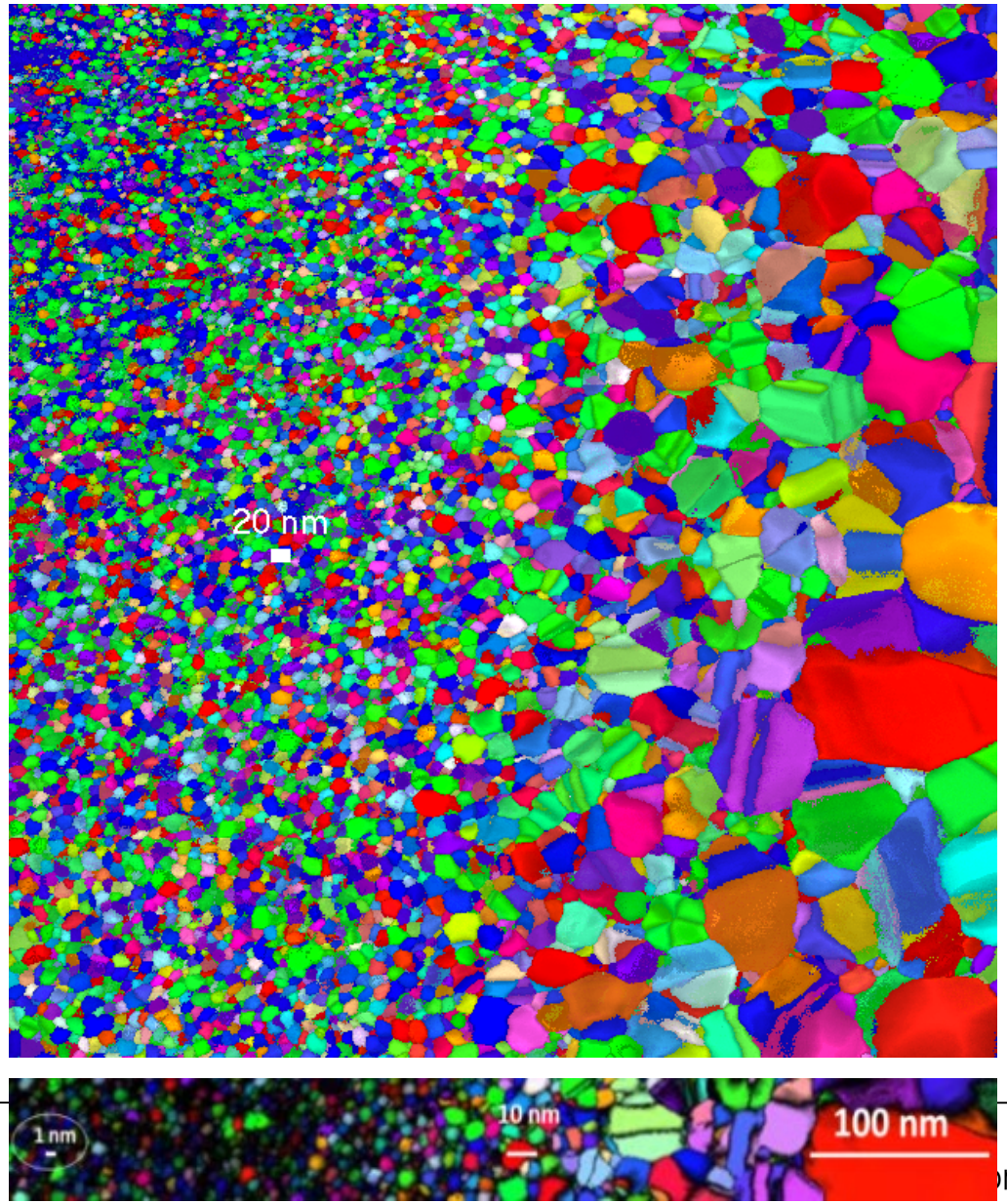
www.nanomegas.com

# ASTAR : Spatial Resolution

**ASTAR**

**From**

**1 nm**
**to**
**500 nm**




**Jeol 2100 FEG-ASTAR**

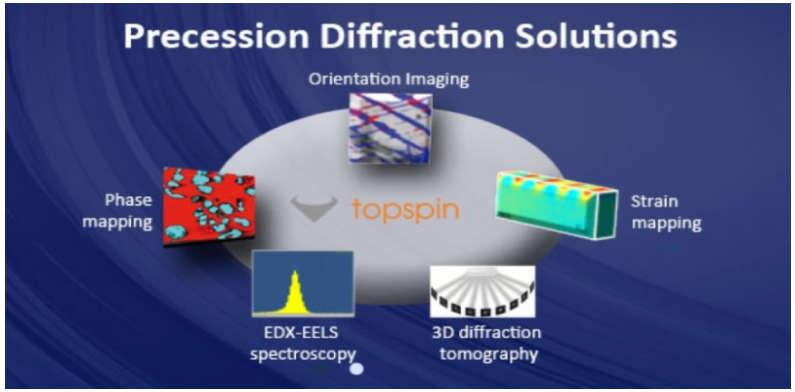
**Seoul Nat University**

**Courtesy Yong -Hwa Oh**
**Muriel Veron Grenoble INP**

**Sample of Cu grains**
**of different sizes**

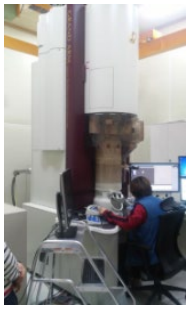
# NanoMEGAS - TEM Cs corr / high end microscopes

TITAN HIGH Base

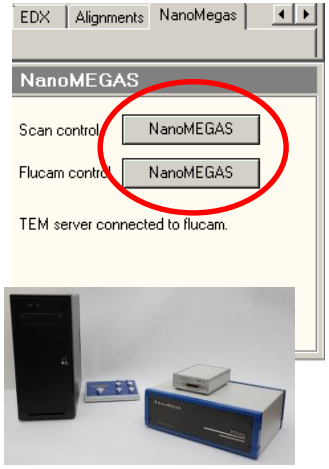



> 400 PED related articles in 12 years
> 180 installations worldwide

ARM 200 FEG Double Cs



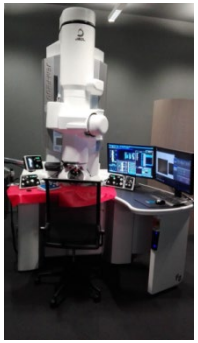
ARM 300 Double Cs




TITAN LOW Base

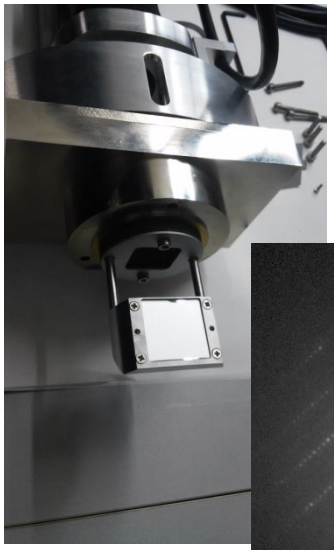

TALOS

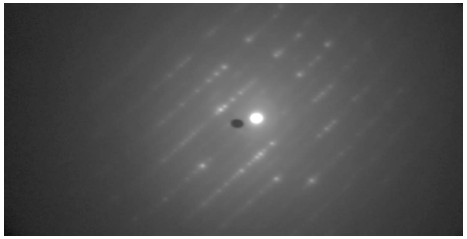
HF5000

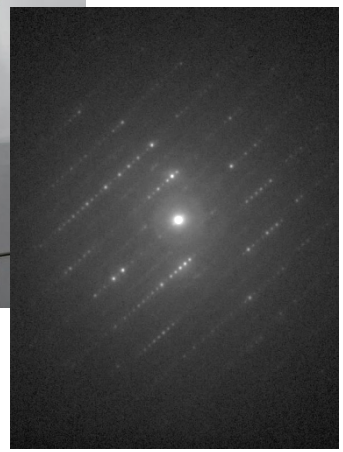


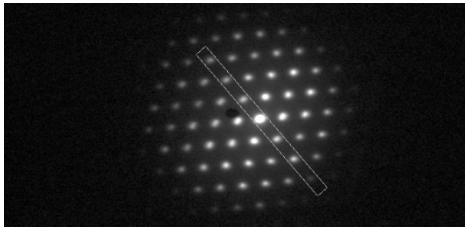
F200

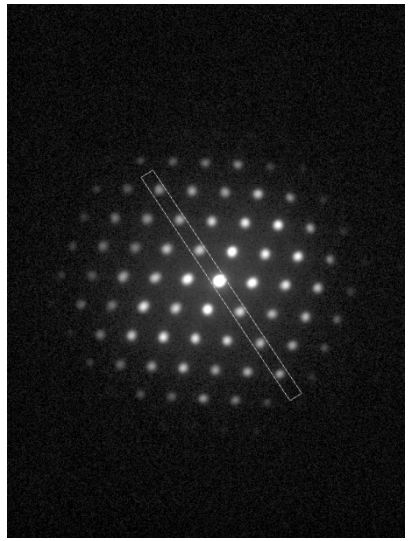
# Comparison Stingray AVT with EMSIS camera support at 35 mm port of JEOL 2010F

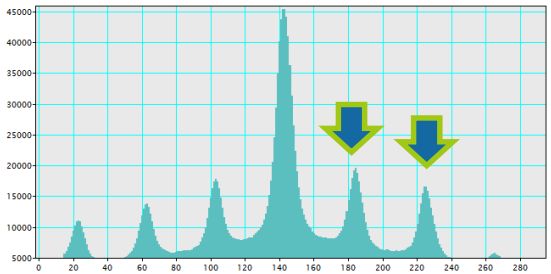


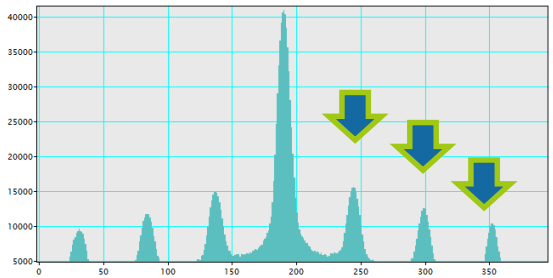






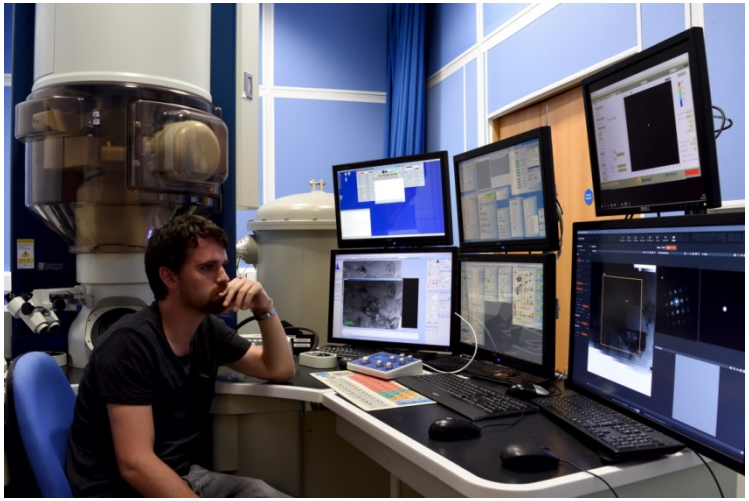


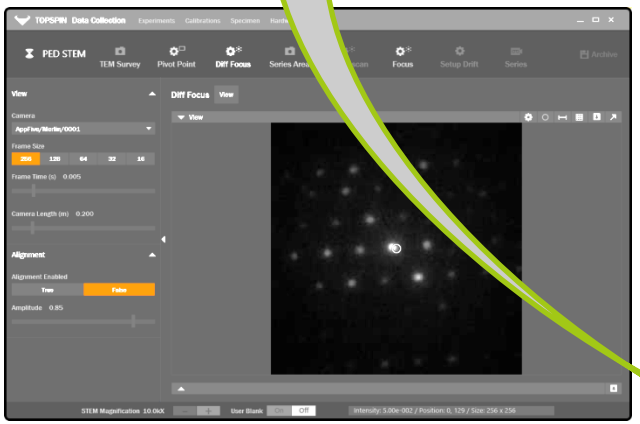


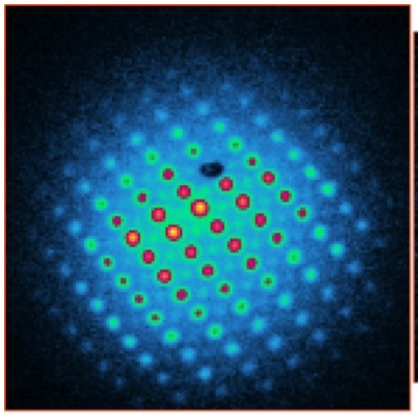



# ASTAR and Direct Detection Medipix III

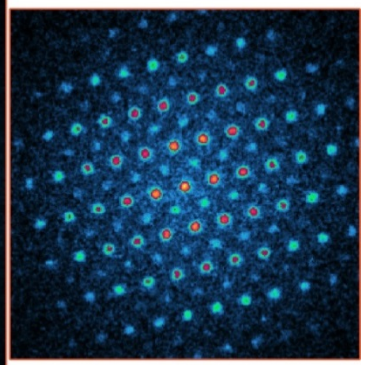
**ASTAR –Topspin**
**Medipix III Merlin**
**Glascow University UK**



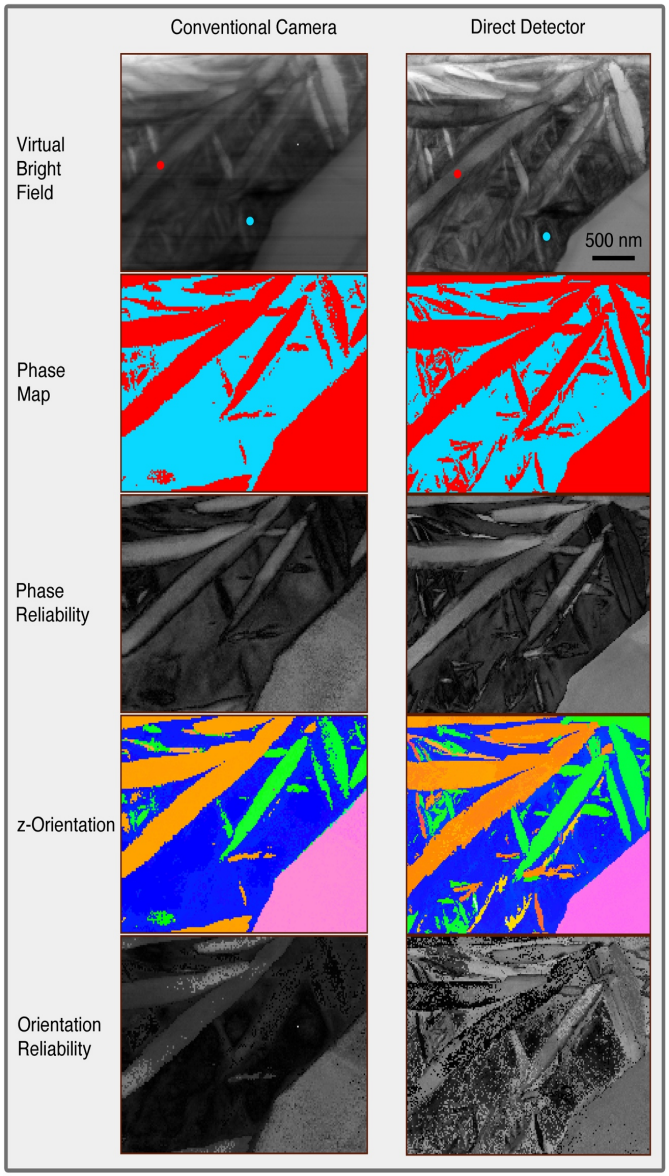



Stingray

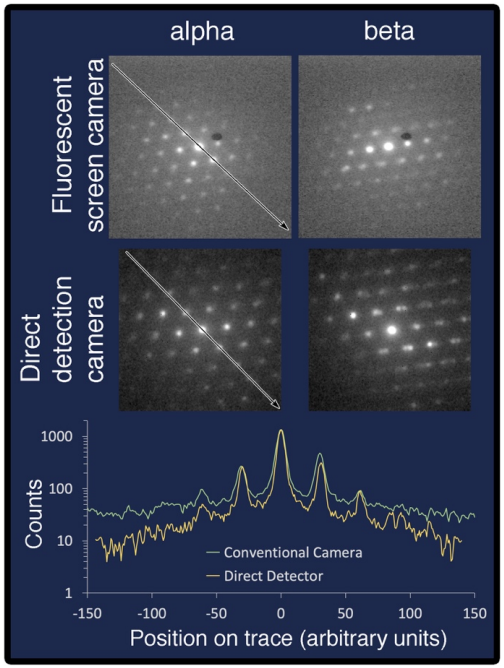


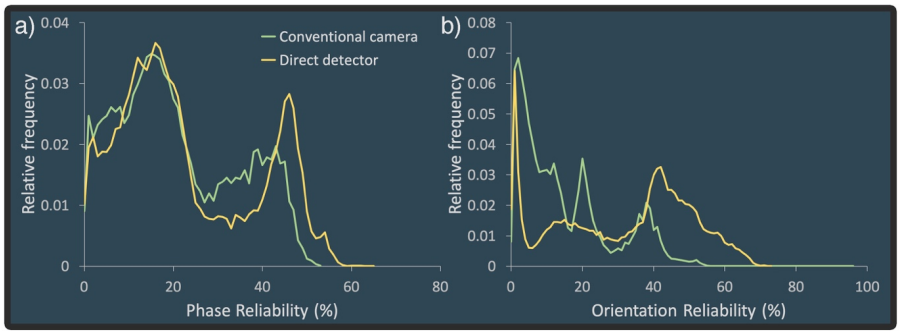
Medipix

# ASTAR and Direct Detection Medipix III




**Ian McLarren et al Submitted for publication**

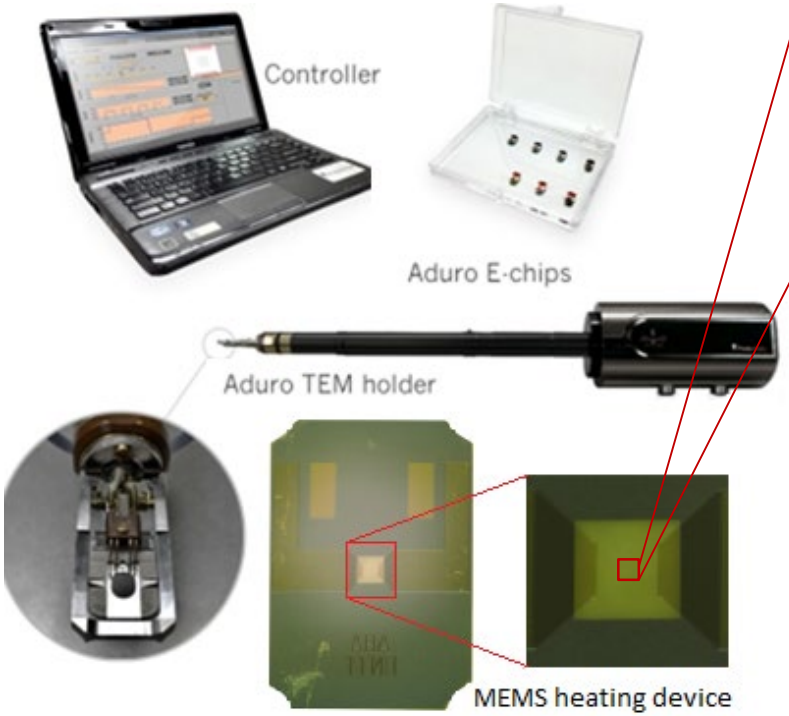


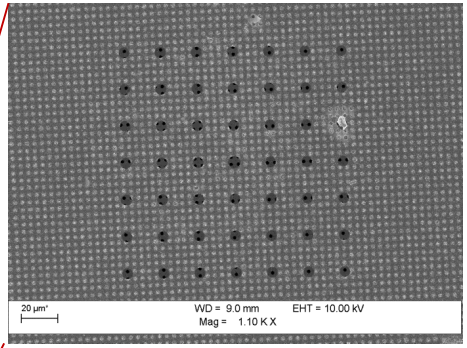



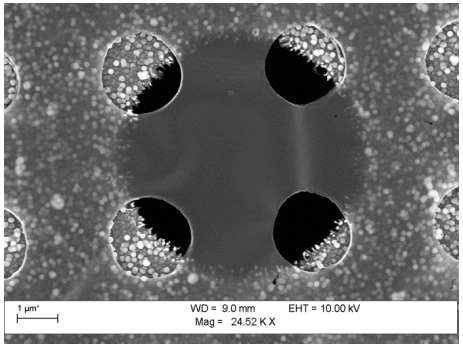
# ASTAR : IN SITU EXPERIMENTS

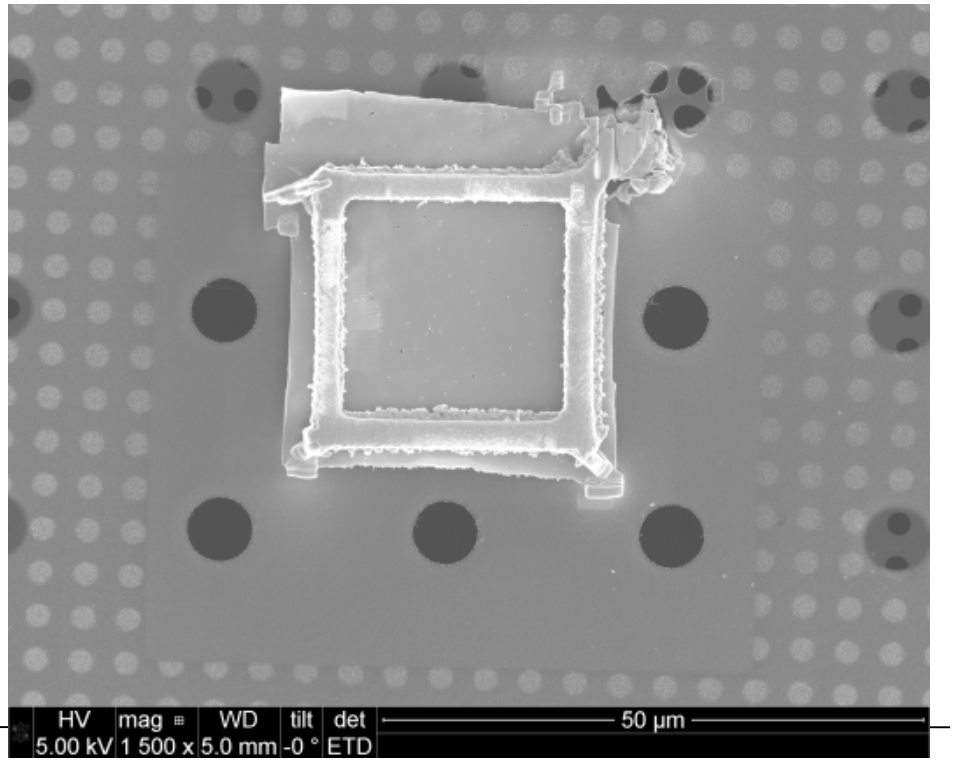
# ASTAR - in situ HEATING of nc Pd

**Protochips Aduro Device and Heating holder**



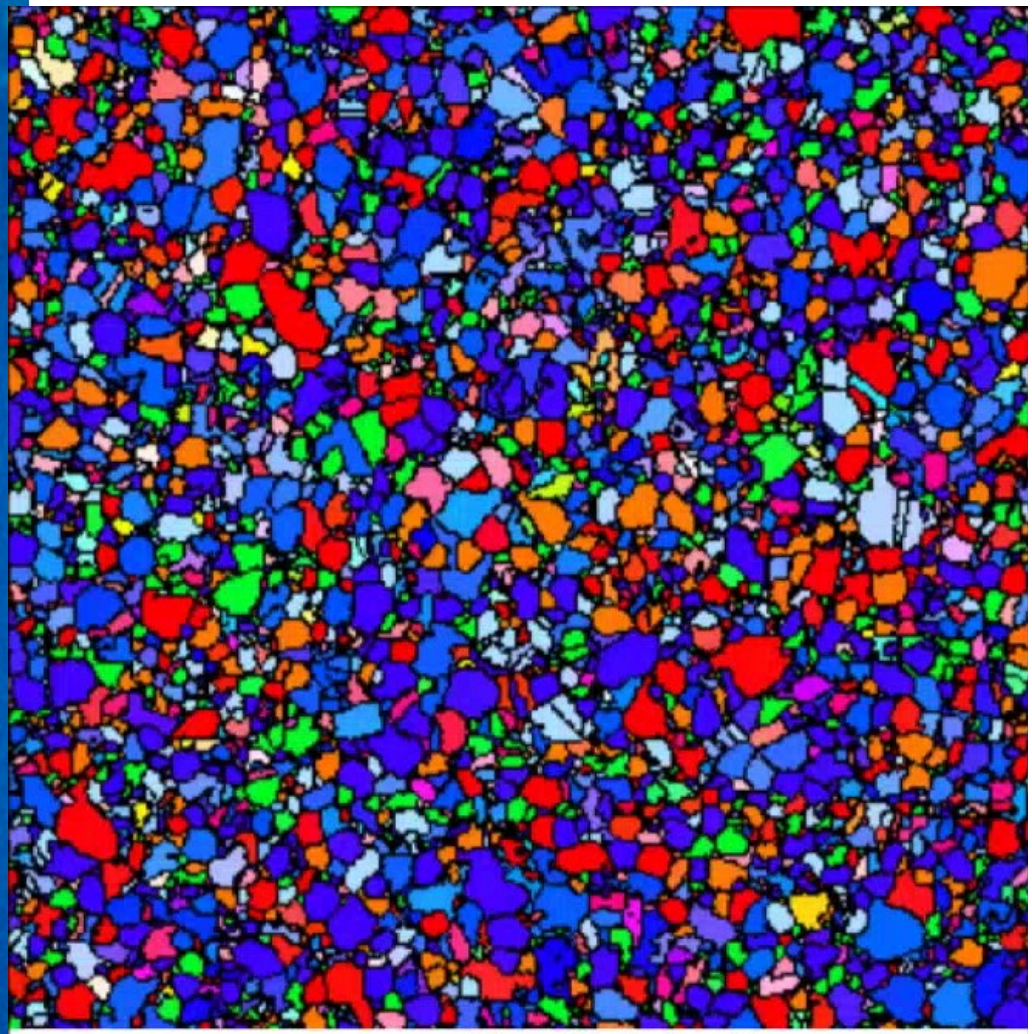



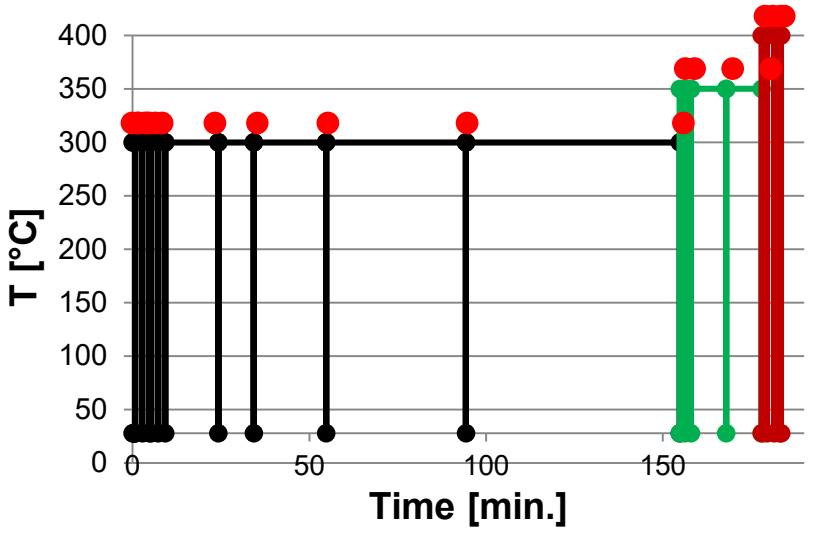



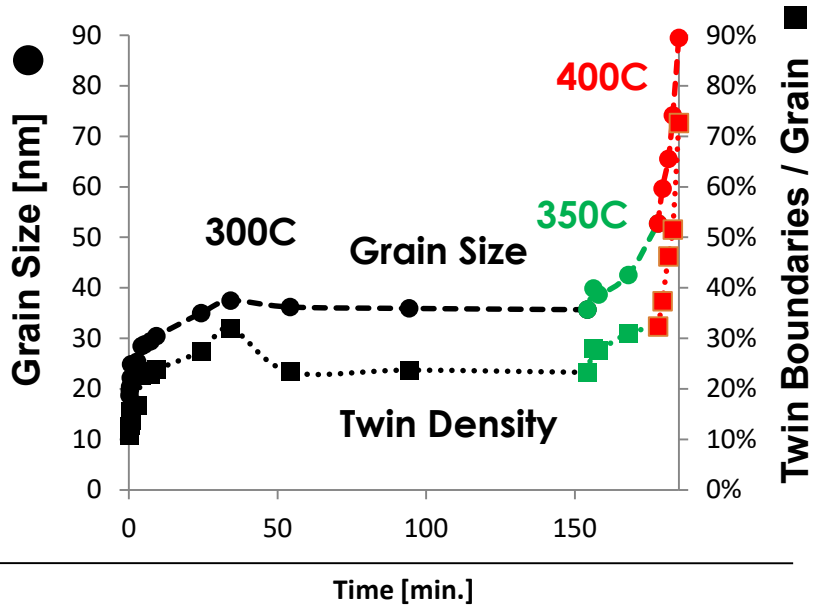




- Fast heating rates ~$10^6$ K/s
- Stable

# ASTAR - in situ HEATING of nc Pd



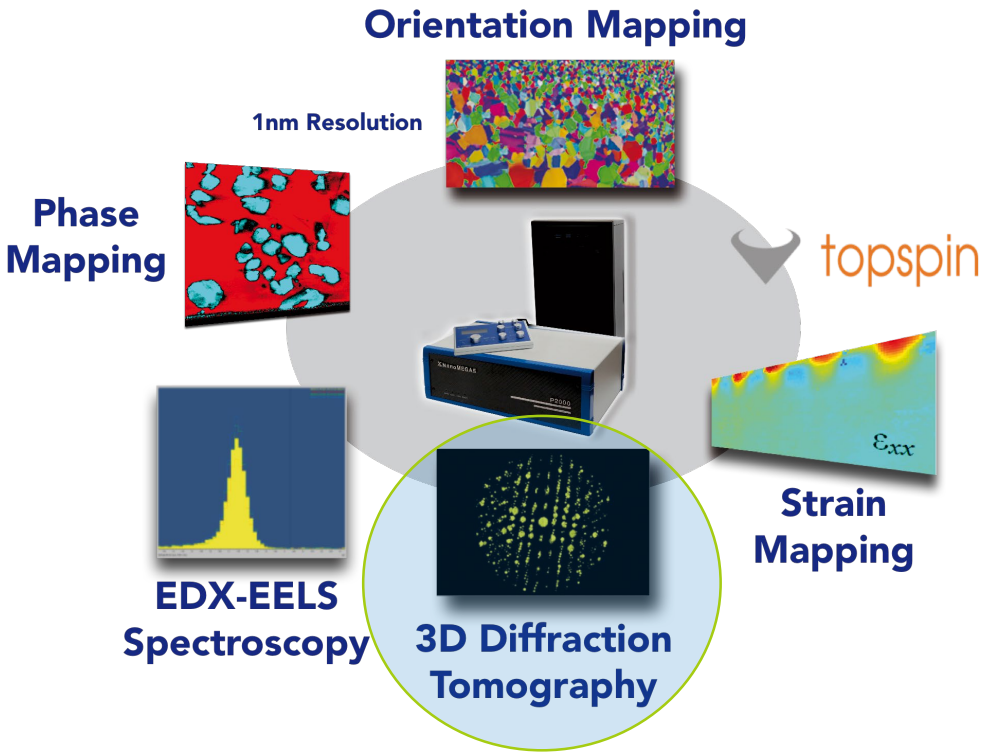

NanoMEGAS
Advanced Tools for electron diffraction

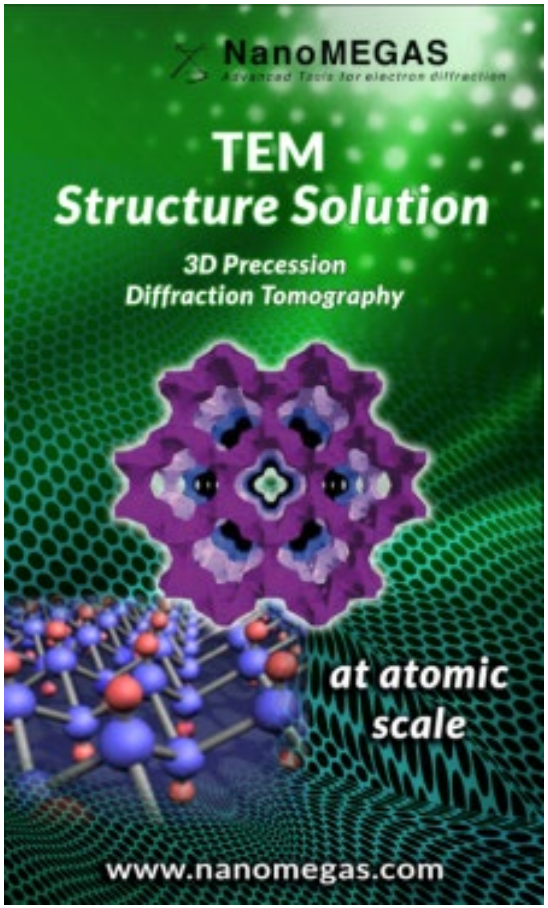
Aaron Kobler, Krishna Kanth Neelisetty, Kiran Chakravadhanula, Christian Kübel, unpublished results

www.nanomegas.com

# 3D DIFFRACTION TOMOGRAPHY AND PIXELATED DETECTORS

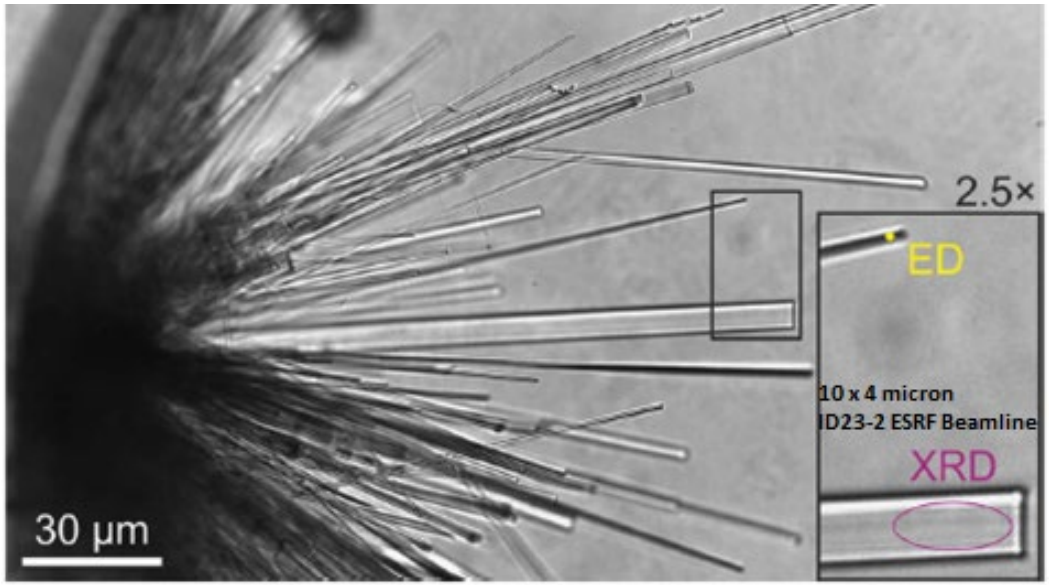
# 3D Diffraction Tomography
# TEM Precession Electron Diffraction



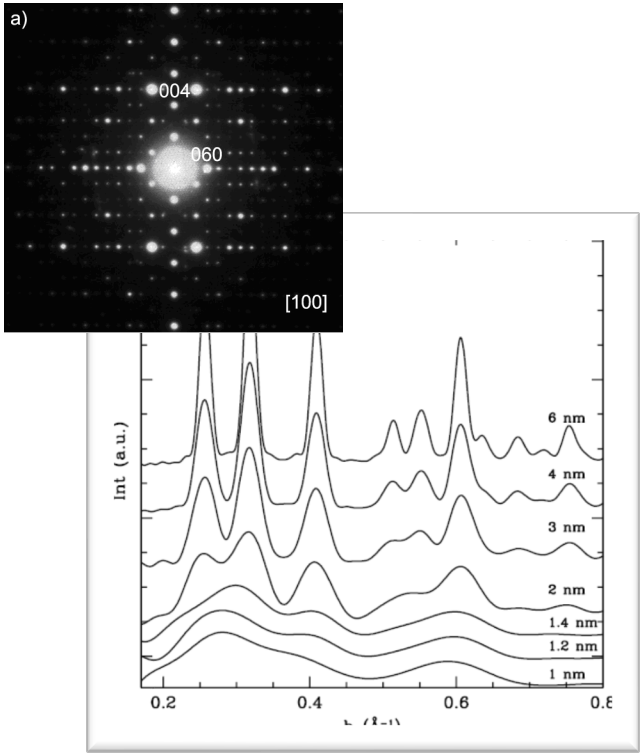



# Why use electrons?

HREM image and Electron diffraction can be taken from areas as small as 50 nm ; Synchrotron smallest beam size 1-4 μ



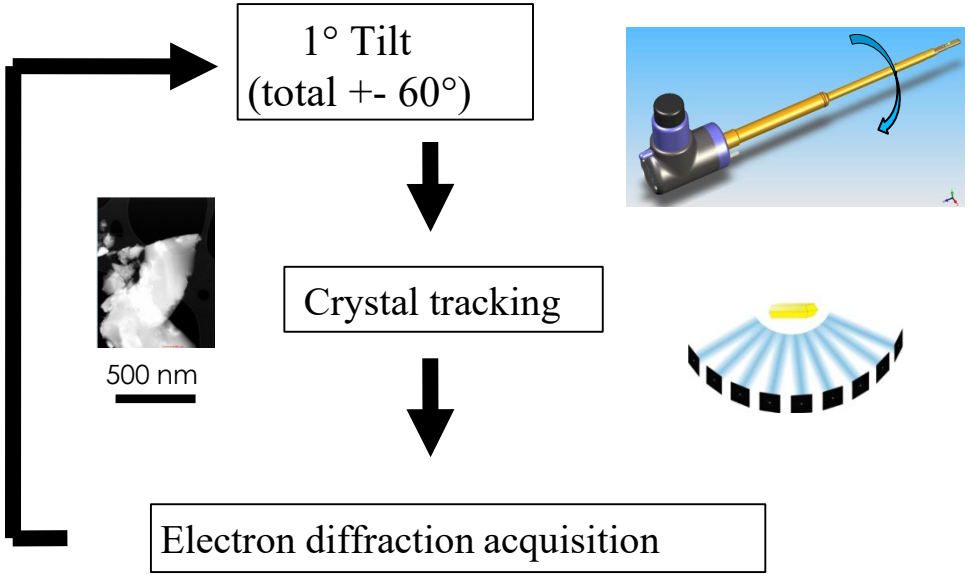

The inset shows two crystals suitable for X-ray diffraction and ED ; Purple ellipse represents the 10 × 4 μm beam of the microfocus X-ray diffraction beamline at ESRF; the yellow dot is four times larger than the diameter of the TEM nanobeam (Lanza et al (2019). IUCrJ, 6, 178-188)

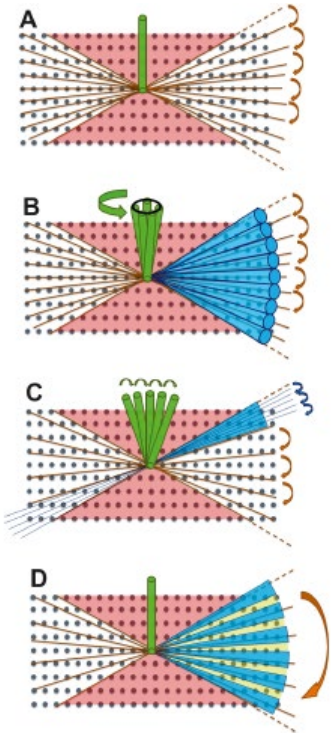



**X-Ray peaks broaden with crystals of nm range**

# 3D Diffraction Tomography

**3D TEM diffraction tomography : 3D reconstruction of reciprocal space**

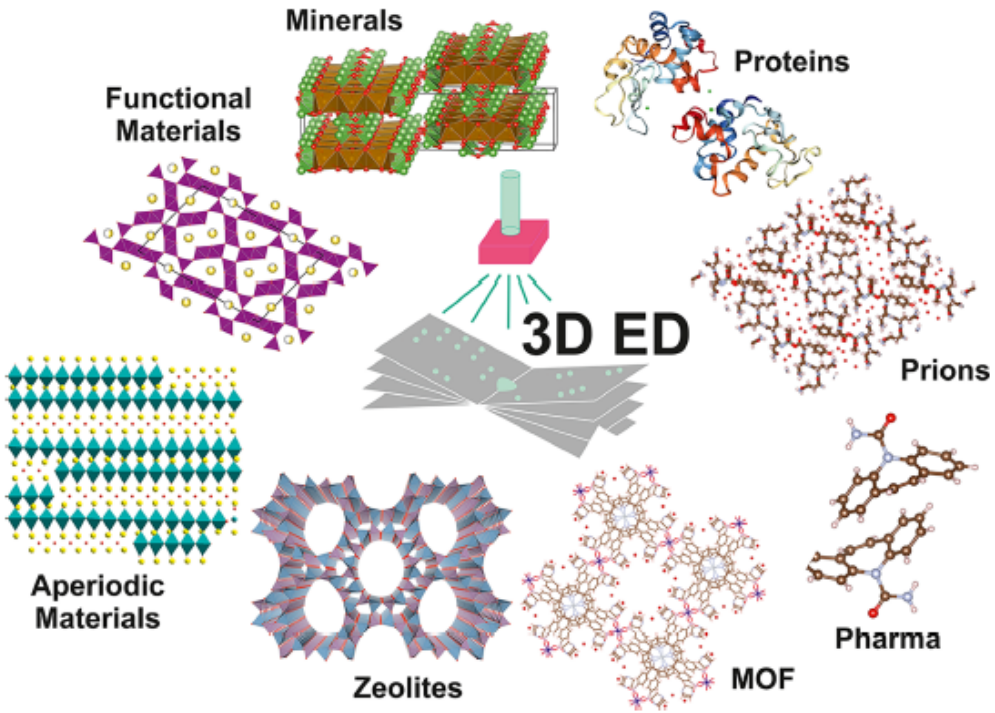



- ✓ complete or almost complete diffraction data to **extract unit cell and crystal symmetry**
- ✓ conceptually simple, data can be taken **with any CCD camera**
- ✓ solution of structures by direct methods or simulated annealing

**R close to 15-35 % : reveal all 3D atomic positions with 5-30 pm precision !**

# > 300 structures solved with Electron Diffraction (2004 - 2019)

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## 3D Electron Diffraction: The Nanocrystallography Revolution

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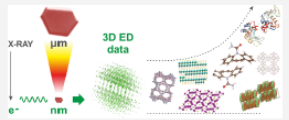
**ABSTRACT:** Crystallography of nanocrystalline materials has witnessed a true revolution in the past 10 years, thanks to the introduction of protocols for 3D acquisition and analysis of electron diffraction data. This method provides single-crystal data of structure solution and refinement quality, allowing the atomic structure determination of those materials that remained hitherto unknown because of their limited crystallinity. Several experimental protocols exist, which share the common idea of sampling a sequence of diffraction patterns while the crystal is tilted around a noncrystallographic axis, namely, the goniometer axis of the transmission electron microscope sample stage. This Outlook reviews most important 3D electron diffraction applications for different kinds of samples and problematics, related with both materials and life sciences. Structure refinement including dynamical scattering is also briefly discussed.




### 1. INTRODUCTION

Accelerated electrons have been long considered the less promising among the radiation types used in crystallography for attaining diffraction data suitable for atomic structure determination. In fact, the large majority of structural models deposited in crystallographic databases[1−3] have been obtained by means of X-ray diffraction, and most of what is left has been derived from neutron diffraction or spectroscopic methods. Although still limited, the use of electron diffraction has grown rapidly over the past decade, mostly due to the introduction of 3D methods for the systematic acquisition and analysis of diffracted intensities. Here, we would like to examine how the use of parallel beam electron diffraction for structure determination has evolved from a mostly qualitative technique, used only by few specialists, to a quantitative approach accessible to a much larger community.

To understand the full picture of this (r)evolution, it is important to focus on the strengths of accelerated electrons for crystallography. First, the possibility to have parallel electron probes with a size of a few nanometers allows collecting diffraction data from sample volumes 2 or 3 orders of magnitude smaller than the ones suitable for synchrotron microfocused X-ray beams. Second, the ability to deliver both diffraction and imaging from the same nanovolume allows the combination of reciprocal and direct space information and the experimental determination of crystallographic phases. Third, the strong Coulomb interaction between electrons and matter allows a good signal-to-noise ratio even from very thin samples and an easier identification of light atoms, like lithium and hydrogen, when compared with X-rays.

However, the strong scattering of electrons is also the reason why electron diffraction (ED) was disregarded for many years for structure analysis. The occurrence of multiple scattering

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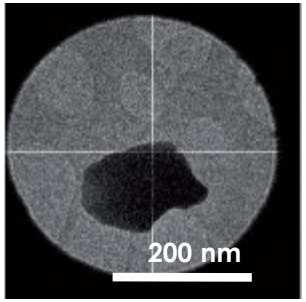
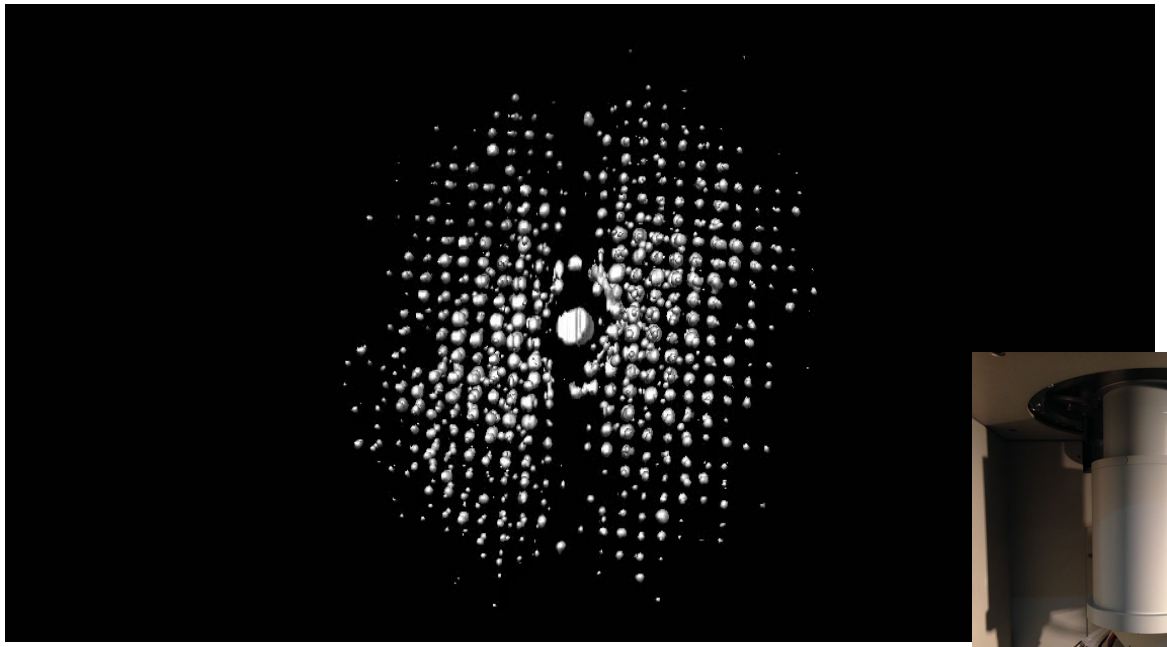
# 3D diffraction tomography of Carbamazepine pharmaceutical crystal

**Low dose with Timepix < 0.013 e-/ Å² /sec**

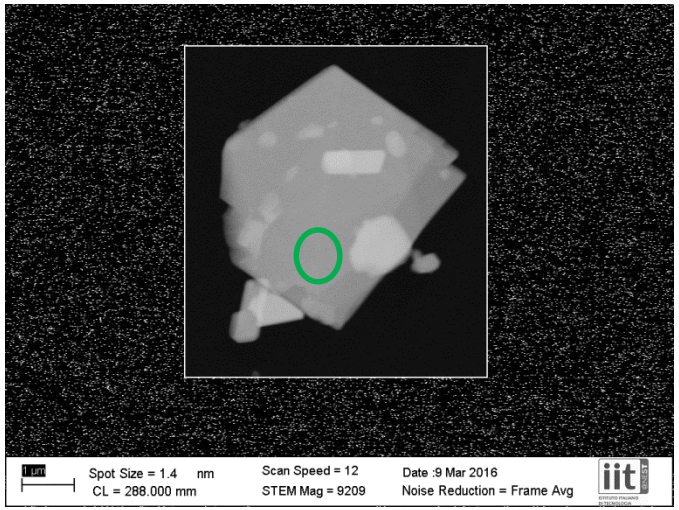


**NO CRYO USED**

**Collection time < 3 min**

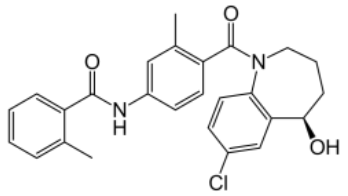
**Diffraction tomography in continuous mode at RT**

# TOLVAPTAN API




**Diffraction tomography in stepwise mode at LT**

-54° to +33°
-40° to +35°
-20° to +38°




API used for autosomal dominant polycystic kidney disease (ADPKD)

**Electron Diffraction Obtained Unit Cell and SPG**

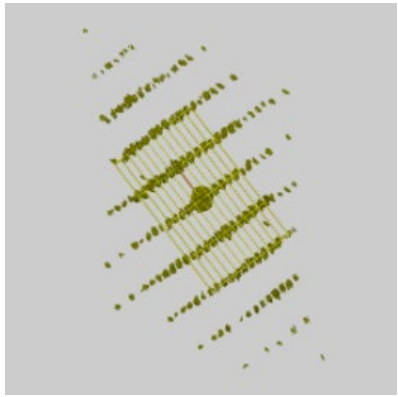
**a = 7.56 Å**
**b = 38.07 Å**
**c = 8.61 Å**
**β = 108.86°**
V=2345.02 Å$^3$

**SPG: P2$_1$/n**

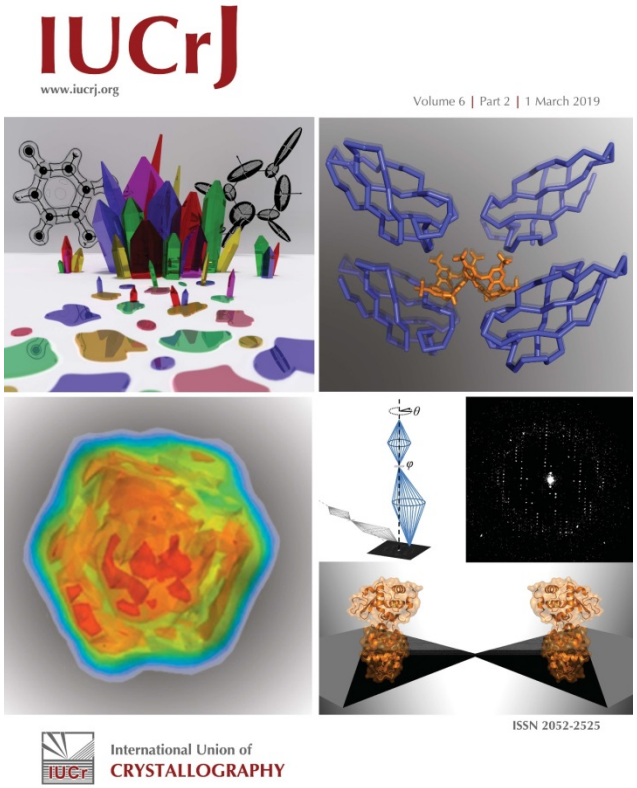
**Single Crystal X-Ray structure**

**a = 7.627(1) Å**
**b = 38.007(4) Å**
**c = 8.5629(12) Å**
**β = 108.118(15)°**
V=2359.16 Å$^3$

**SPG: P2$_1$/n**



# PROTEIN CRYSTAL STRUCTURE - PRECESSION DIFFRACTION TOMOGRAPHY




research papers

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## Nanobeam precession-assisted 3D electron diffraction reveals a new polymorph of hen egg-white lysozyme

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**Keywords:** macromolecular crystallography; electron crystallography; nanobeam precession-assisted electron diffraction; monoclinic lysozyme; radiation damage.

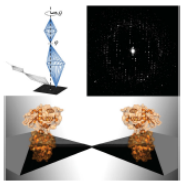
**PDB references:** hen egg-white lysozyme, X-ray microfocus diffraction structure, 6ht2; electron diffraction structure, 6hu5

**Supporting information:** this article has supporting information at www.iucrj.org

Recent advances in 3D electron diffraction have allowed the structure determination of several model proteins from submicrometric crystals, the unit-cell parameters and structures of which could be immediately validated by known models previously obtained by X-ray crystallography. Here, the first new protein structure determined by 3D electron diffraction data is presented: a previously unobserved polymorph of hen egg-white lysozyme. This form, with unit-cell parameters $a$ = 31.9, $b$ = 54.4, $c$ = 71.8 Å, $\beta$ = 98.8°, grows as needle-shaped submicrometric crystals simply by vapor diffusion starting from previously reported crystallization conditions. Remarkably, the data were collected using a low-dose stepwise experimental setup consisting of a precession-assisted nanobeam of ~150 nm, which has never previously been applied for solving protein structures. The crystal structure was additionally validated using X-ray synchrotron-radiation sources by both powder diffraction and single-crystal micro-diffraction. 3D electron diffraction can be used for the structural characterization of submicrometric macromolecular crystals and is able to identify novel protein polymorphs that are hardly visible in conventional X-ray diffraction experiments. Additionally, the analysis, which was performed on both nanocrystals and microcrystals from the same crystallization drop, suggests that an integrated view from 3D electron diffraction and X-ray microfocus diffraction can be applied to obtain insights into the molecular dynamics during protein crystal growth.

### 1. Introduction

In order to address many challenging scientific issues concerning structural biology, several X-ray microfocus beamlines worldwide are fully dedicated to the analysis of 3D protein crystals smaller than a few tens of micrometres (Smith *et al.*, 2012). The relevance of nanocrystallography is driving some beamlines to achieve beam sizes below 1 µm in order to investigate even smaller protein crystals (Moukhametzianov *et al.*, 2008; Owen *et al.*, 2016). However, access for the scientific community to X-ray microfocus beamlines or to unconventional approaches such as X-ray free-electron lasers (XFELs; McNeil & Thompson, 2010) is strongly limited (Grimes *et al.*, 2018), and therefore there is growing interest in the development of alternative approaches. In this regard, electron-microscopy methods appear to be particularly promising. Cryo-EM imaging has rapidly become a widespread technique that is able to skip the crystallization step by directly imaging macromolecular complexes or potentially even single molecules of sufficient size (Amunts *et al.*, 2014; Kühlbrandt, 2014; Cheng, 2015; Nogales & Scheres, 2015; Fernandez-Leiro & Scheres, 2016; Quentin & Raunser, 2018).



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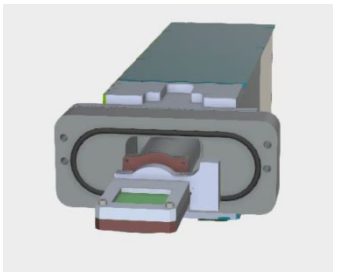

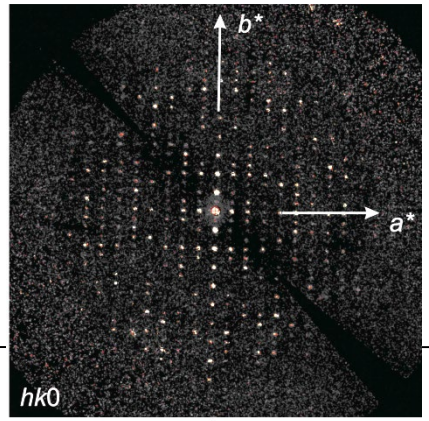


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**THANK YOU !!!**